

I CLAIM:

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A method for treatment for pythiosis or prophylaxis against pythiosis which comprises:

5 (a) providing a vaccine consisting of intracellular cytoplasmic antigens separated from disrupted cells of *Pythium insidiosum* and extracellular antigens secreted into a medium for growing the cells of the *Pythium insidiosum*. in a sterile aqueous solution; and

(b) vaccinating the patient with the vaccine.

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The method of Claim 1 wherein the vaccination is subcutaneous.

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5 The method of Claim 1 wherein the patient after vaccination is monitored for a change in a Th1 response and a Th2 response, wherein an increase in the Th1 response and a decrease in the Th2 indicates the patient has developed the Th1 response to the vaccine.

A method for treatment of pythiosis or prophylaxis against pythiosis in a mammal which comprises:

5 (a) providing an injectable vaccine which comprises in a sterile aqueous solution in admixture:

(i) intracellular cytoplasmic antigens separated from disrupted cells of *Pythium insidiosum*; and

10 (ii) extracellular antigens secreted into a medium for growing the cells of the *Pythium insidiosum*; and

(b) vaccinating the mammal with the vaccine.

The method of Claim 4 wherein the antigens have been provided by

(a) growing cells of the *Pythium insidiosum* in a culture medium and then

5 (i) killing the cells;

(ii) separating the killed cells from the culture medium so as to produce a first supernatant comprising the extracellular antigens secreted into the medium; and

10 (ii) disrupting the cells in water to provide the intracellular cytoplasmic antigens in a second supernatant which is separated from the disrupted cells; and

15 (b) separating the extracellular antigens from the first supernatant.

The method of Claim 4 wherein the cells have been disrupted by sonication.

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The method of Claim 4 wherein the *Pythium insidiosum* is deposited as ATCC 74446.

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The method of any one of Claims 5, 6, or 7 wherein the culture medium is Sabouraud dextrose broth.

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The method of Claim 5 wherein the cells are killed with thimersol.

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The method of Claim 5 wherein the disrupted cells are separated from the culture medium for the cells by centrifugation.

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5 The method of Claim 5 wherein the intracellular cytoplasmic antigens in the second supernatant and the extracellular antigens in the first supernatant are mixed to provide a mixture of antigens, precipitating the mixture of antigens with acetone to provide a precipitate, dissolving the precipitate in sterile distilled water to provide a solution of the antigens, and dialyzing the solution of antigens in
10 sterile distilled water to remove low molecular weight components less than 10,000 MW to provide the vaccine.

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5 The method of Claim 4 wherein the mammal after vaccination is monitored for a change in a Th1 response and a Th2 response, wherein an increase in the Th1 response and a decrease in the Th2 indicates the patient has developed the Th1 response to the vaccine.

A method for providing an injectable vaccine for treatment of pythiosis or prophylaxis against pythiosis which comprises:

5 (a) growing cells of *Pythium insidiosum* in a culture medium;

(b) separating the cells from a first supernatant of the culture medium which contains extracellular proteins;

(c) killing the cells;

10 (d) disrupting the cells in sterile water;

(e) separating the disrupted cells from the water to produce a second supernatant containing intracellular proteins;

15 (f) mixing the first supernatant of step (b) with the second supernatant of step (e);

(g) separating the combined proteins from the mixture of step (f);

(h) mixing the separated proteins in sterile distilled water; and

20 (i) dialyzing the mixture of step (h) to remove low molecular weight components less than 10,000 MW to produce the vaccine.

The method of Claim 13 wherein the cells are disrupted by sonication.

The method of Claim 13 wherein the *Pythium insidiosum* is deposited as ATCC 74446.

The method of any one of Claims 13, 14, or 15 wherein the culture medium is Sabouraud's dextrose broth.

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The method Claim 13 wherein the cells are killed with thimersol.

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The method Claim 13 wherein the disrupted cells are separated from the water in step (e) by centrifugation.

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The method of Claim 13 wherein the separated proteins are separated in step (g) by being precipitated together using acetone from the first and second supernatants combined together.

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A method of testing a response in a mammal to a *Pythium insidiosum* vaccine which comprises:

monitoring a Th1 response and a Th2 response of the mammal to the vaccine, wherein in mammals which are responding to the vaccine the Th1 response increases and the Th2 response decreases.

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The method of Claim 20 wherein the vaccine comprises antigens selected from the group consisting of intracellular cytoplasmic antigens separated from disrupted cells of *Pythium insidiosum*, extracellular antigens secreted into a medium for growing the cells of the *Pythium insidiosum*, and combination of both.

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The method of Claim 21 wherein the antigens have been provided by growing cells of the *Pythium insidiosum* in a culture medium and then killing the cells, separating the killed cells from the culture medium so as to produce a first supernatant comprising the extracellular antigens secreted into the medium, separating the extracellular antigens from the first supernatant, and disrupting the cells in water to provide the intracellular cytoplasmic antigens in a second supernatant which is separated from the disrupted cells.

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The method of Claim 21 wherein the *Pythium insidiosum* is deposited as ATCC 74446.

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The method of Claim 20 wherein the mammal is infected with the *Pythium insidiosum* and the vaccine is for immunotherapy.

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The method of Claim 20 wherein the mammal is not infected with the *Pythium insidiosum* and the vaccine is for prophylaxis.

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A mammal model for testing a *Pythium insidiosum* vaccine which comprises:

monitoring a Th1 response and a Th2 response of the mammal in the mammal model to the vaccine, wherein in mammals which are responding to the vaccine the Th1 response increases and the Th2 response decreases.

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5 The mammal model of Claim 26 wherein the vaccine comprises antigens selected from the group consisting of intracellular cytoplasmic antigens separated from disrupted cells of *Pythium insidiosum*, extracellular antigens secreted into a medium for growing the cells of the *Pythium insidiosum*, and combination of both.

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5 The mammal model of Claim 27 wherein the antigens have been provided by growing cells of the *Pythium insidiosum* in a culture medium and then killing the cells, separating the killed cells from the culture medium so as to produce a first supernatant comprising the extracellular antigens secreted into the medium, separating the extracellular antigens from the first supernatant, and disrupting the cells in water to provide the intracellular cytoplasmic antigens in a
10 second supernatant which is separated from the disrupted cells.

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The mammal model of Claim 27 wherein the *Pythium insidiosum* is deposited as ATCC 74446.

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The mammal model of Claim 26 wherein the mammal in the mammal model is a rabbit.

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The mammal model of Claim 26 wherein the mammal is infected with the *Pythium insidiosum* and the vaccine is for immunotherapy.

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The mammal model of Claim 26 wherein the mammal is not infected with the *Pythiosum insidiosum* and the vaccine is for prophylaxis.